



Applicants : Seetharama A. Acharya and Belur N. Manjula

Appn. No. : 10/538,976

I.A. Filing Date : December 5, 2005

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Amendments to the Specification:

Please replace the current first paragraph in the application with the following replacement paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a U.S. national stage entry under 35 U.S.C. §371 of PCT International Patent Application No. PCT/US2003/040407, filed on December 18, 2003, and claims priority to U.S. Provisional Patent Application No. 60/436,149, filed on December 23, 2002.

Please add the following new paragraph on page 1 after the first paragraph:

STATEMENT OF GOVERNMENT SUPPORT

[0002] The invention disclosed herein was made with U.S. Government support under grant number HL71064 from the National Institutes of Health and grant number PR023085 from the U.S. Army. Accordingly, the U.S. Government has certain rights in this invention.

Please amend paragraph [0005] on pages 2-3 as follows:

[0005] The demonstration that Enzon polyethylene glycolylated (PEGylated) Hb, that carries ten copies of PEG-5000 chains linked to Hb at its  $\alpha$  and  $\epsilon$ -amino groups is non-hypertensive has stimulated the research in the blood substitute field (Rolfs et al. 1998). The NO binding activity of intra-tetramERICALLY crosslinked Hbs, oligomerized Hbs and PEGylated Hbs (Winslow et al, 1998, Vandegriff et al, 1997) do not show a direct correlation with their 'pressor effects'. Thus, the reduction in the 'pressor activity' of acellular Hb does not appear to be a direct correlate of, either the NO binding activity of the preparation or of the molecular size of the preparation. But the PEGylated Hbs exhibited considerably lower level of vasoactivity as compared to the oligomerized Hb. The PEG-Bv-

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Hb of Enzon that carries 10 copies of PEG-5,000 exhibited hardly any 'pressor effect'. Vandegriff et al (1998) have noted that PEG-Bv-Hb exhibited high viscosity and oncotic pressure as compared to that of oligomerized samples of Hb. The molecular radius of Enzon PEGylated Bv-Hb calculated from the oncotic pressure was considerably larger (15 nm) than that of oligomerized Hbs and the molecular radius calculated is not consistent with its calculated molecular mass of 114,000 daltons (Vandegriff et. al. 1998). Accordingly, it has been hypothesized that size of Hb should be increased to a molecular radius of around 15 nm, and this should be accompanied by considerable increase in the viscosity and oncotic pressure to generate a non-hypertensive Hb solution (Winslow 1999).

Please replace Figures 2A, 3 and 8 with Replacement Figures 2A, 3 and 8 attached hereto (3 sheets).